REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks. Applicants are grateful for the numerous telephonic discussions with the Examiner. The foregoing amendment and the following remarks address the concerns discussed during these discussions.

I. CLAIM STATUS & AMENDMENTS

Claims 12-27, 29, and 31-37 are pending in this application, and stand rejected.

The present amendment amends claim 12-14.

Applicants reserve the right to file a continuation or divisional application on any canceled subject matter.

Support for recitations "effective against and specific for herpes group virus infections" can be found in the Specification, for example, at page 6, line 23 to page 7, line 2, at page 8, lines 6-9, and at page 13, lines 14-15.

Thus, no new matter has been added by this amendment.

II. REJECTION UNDER 35 U.S.C. § 102/103

Claims 12, 15-18, 29, 33, and 35 stand rejected under 35 U.S.C. § 102(b), as allegedly anticipated by, or in the alternative, under 35 U.S.C. § 103(a), as allegedly obvious over Koenig. See Office Action, pages 2-3. Applicants respectfully traverse this rejection as applied to the amended claims and for the reasons noted below.

Koenig fails to anticipate the claimed invention because the cited prior art reference fails to teach or suggest each and every element of the claimed invention, namely "activated autologous lymphocytes effective against and specific for herpes group viral infections" as claimed.

Koenig also lacks a suggestion and/or motivation to modify its teaching to arrive at the claimed invention. Koenig also lacks a reasonable expectation of success given the teaching away in the prior art.

The claims call for activated autologous lymphocytes effective against and specific for herpes group viral infections. These lymphocytes are derived from the same herpes virus infected patient who ultimately undergoes treatment with such lymphocytes. As such, they are autologous lymphocytes and they are ***virus-specific*** for herpes group viral infections.

Koenig fails to teach or suggest this. Instead, Koenig is related to HIV-1-specific cytotoxic T lymphocytes obtained from an AIDS patient, activated with OTK-3 and IL-2 and re-injected into the patient. Consequently, such lymphocytes are ***specific for HIV-1 infections, not herpes group viral infections***. These lymphocytes would not have the specificity of the present invention, because they would induce a HIV-1-specific immune reaction.

During discussions with the Examiner, Applicants directed the Examiner's attention to the fact that the prior art fails to teach lymphocytes having the same specificity of those in the present invention. In other words, the lymphocytes of the present invention have a specificity for herpes virus, whereas the lymphocytes of the cited prior art have a specificity for cancer and HIV. The claims are directed to activated lymphocytes derived from a herpes virus infected patient which are then used to treat the patient. Consequently, the activated lymphocytes are effective for and specific to herpes virus infection.

In support of this position, Applicants again direct the Examiner's attention to the reference article by Numazaki et al. (Clinical Infectious Diseases, Vol. 25, No. 5, pp. 1246-1247 (Nov. 1997)) entitled "Adoptive Immunotherapy for Interstitial Pneumonia Associated with Cytomegalovirus Infection." This reference was previously submitted with the response dated January 13, 2003. At page 1246, 2nd column, line 3 and at page 1247, 2nd column, line 11, the reference discloses using "antigen-specific cytotoxic T lymphocytes" and "virus-specific CD8+ cytotoxic cells" to treat certain herpes group viral infections. The terms ***antigen-specific*** and

virus-specific show that the activated T lymphocytes are specific to the virus that infected the patient from which the cells were derived. This article also demonstrates that the experimental results directed to the Epstein-Barr and herpes simplex viruses support the effectiveness of the present invention for herpes group viral infections.

In further support, Applicants direct the Examiner's attention to the copies of two reference articles which are enclosed herewith together with their English translations, Kawata, Journal of Japan Pediatric Society, Vol. 106, No. 3, pp. 411-412 (2002) and Ayumi (Journal of Clinical and Experimental Medicine, Vol. 181, No. 6 (May 1997). These article demonstrate the effectiveness of the claimed invention for inducing a **virus-specific** immune response for herpes group virus infections. In other words, the activated autologous cytotoxic T lymphocytes are virus-specific. See Ayumi, page 3, lines 7-8 and Kawata, page 2, lines 21-27.

In view of these articles, it becomes clear then that the HIV-1-specific cytotoxic T lymphocytes obtained from an AIDS patient as disclosed in Koenig are virus-specific for HIV infections, and not herpes group viral infections.

Furthermore, to render the claimed invention obvious, Koenig must contain a suggestion to modify the lymphocytes so that they are effective against and specific for herpes virus infections. However, nothing in Koenig discloses or alludes to their being effective against any other viral infection, especially herpes virus infections. Koenig lacks a suggestion that activated autologous lymphocytes as recited in the present invention would be effective against herpes viral infections. As a result, Koenig does not create a reasonable expectation of success that activated autologous lymphocytes derived from a HIV-1 infected patient would be very effective for herpes virus infections or that the same techniques could be applied to herpes virus infections.

Moreover, it is well established that herpes viruses and the HIV virus are vastly different viruses with different physical properties and different pathologies.

Given these vast differences, one of ordinary skill in the art would expect lymphocytes derived from a HIV-1 infected patient to be specific for HIV-1 viruses, whereas those derived

from a herpes virus infected patient to be specific for herpes viruses. One of ordinary skill in the art would not expect lymphocytes derived from a HIV-1 infected patient to be effective for a herpes virus patient.

In fact, as discussed in Applicant's response of January 13, 2003, the prior art contained a ***teaching away*** from the claimed invention. The reference, Nature Medicine, 1(4) pp. 330-336 (1996), discloses that lymphocytes prepared using IL-2 ***were not effective*** in remedying HIV infections, as stated on page 4 of the instant Specification.

At the 4th paragraph on page 4 of the Office Action, the Examiner dismissed the Nature Medicine article as being only a single article that is limited to HIV, and further argued that it did not appear to conclude that treatment with autologous lymphocytes would not be effective in treating viral infections. While this article may not rule out the use of autologous lymphocytes in treating viral infections, it clearly states that these cells were ineffective in remedying HIV infections. In doing so, it casts doubt on and conflicts with the teaching in Koenig. Thus, the skilled artisan, upon reading the Nature Medicine article and Koenig, would certainly not have a reasonable expectation of successfully using the technique in Koenig for HIV-1 infections to treat herpes virus infections.

Given this teaching away and the above-noted differences between herpes viruses and HIV viruses, one of ordinary skill in the art would not reasonably expect lymphocytes derived from a HIV-1 infected patient to be effective for a herpes virus patient, nor that this technique would be effective for herpes virus infections. Koenig and the prior art in general, as evidenced by the Nature Medicine article, simply lack a reasonable expectation of success.

Thus, Koenig fails to teach or suggest the claimed invention. For this reason, Koenig cannot anticipate or render obvious the claimed invention.

In view of the above, the rejection of claims 12, 15-18, 29, 33, and 35 under 35 U.S.C. § 102(b) and 103 is untenable and should be withdrawn.

III. REJECTION UNDER 35 U.S.C. § 103

Claims 12-27, 29, and 31-37 are rejected under 35 U.S.C. § 103 as unpatentable over Ochoa et al. in view of Rosenberg et al. and Melder et al. and further in view of Wallace et al. and Rooney et al. See Office Action, pages 3-5.

Applicants respectfully traverse this rejection as applied to the amended claims for the same reasons discussed immediately above, and for the reasons set forth below.

The claims are directed to activated autologous lymphocytes effective against and specific for herpes group viral infections.

Ochoa and Rosenberg fail to teach activated ***autologous*** lymphocytes ***effective against*** and ***specific for herpes group viral infections***. In fact, neither Ochoa nor Rosenberg disclose or suggest ***autologous*** lymphocytes.

Autologous lymphocytes are derived from the same patient who ultimately undergoes treatment with the lymphocytes. In Ochoa, the lymphocytes are not derived from the patient to be treated. Ochoa discloses an example in which peripheral blood lymphocytes (PBLs) are collected from the twin brother of a patient, not from the same patient. (see Example 4 of the reference). Twin brothers are identical in genetics, but have ***different*** activated lymphocytes. As such, the derived cells are not autologous. While Ochoa discusses generally the preparation of lymphocytes using anti-CD3 antibody and IL-2, it does not at all teach or suggest the limitation autologous. Furthermore, Ochoa is also limited to a treatment of cancer, not herpes group viral infections. Ochoa only shows a preparation of lymphocytes against tumors and does not at all teach or suggest activated autologous lymphocytes having ***antiviral activity***.

Similarly, Rosenberg is related only to lymphocytes prepared using IL-2 only and does not at all teach or suggest the claim limitation ***autologous***. Rosenberg is not related to activated lymphocytes and their use to treat herpes group viral infections. Instead, Rosenberg is limited to the treatment of cancer.

In column 4, lines 49-54, Rosenberg suggests that activated lymphocytes "can be employed" for the treatment of viral infections. This is merely a suggestion. There is absolutely no evidence to support the effectiveness of such treatment. Moreover, this is not suggestive of activated autologous lymphocytes effective against and specific for herpes group viral infections. It is well established that herpes group viral infections and cancer are vastly different disease conditions with different etiologies and pathologies. As such, one of ordinary skill in the art would ***not*** expect that a treatment for one would be suggestive for the other.

At page 5, lines 14-16 of the Office Action, it is indicated that "one of ordinary skill in the art would expect from the teachings of Melder et al. that the activated lymphocytes, since they are effective against cancer cells, would also be effective against viruses, including herpes group viruses." Applicants respectfully disagree with this unsupported assertion. Again, herpes group viral infections and cancer are vastly different disease conditions, and one of ordinary skill in the art would ***not*** expect that a treatment for one would be suggestive for the other.

The rejection relies on Melder, Wallace, and Rooney for suggesting their use against herpes group viral infections.

Melder relates to how natural killer (NK) cells aid in the control of viral infections. However, the activated autologous lymphocyte cells used in the present invention are distinctly different from NK lymphocyte cells and LAK cells. The activated T lymphocytes according to the present invention have T-cell receptors through which antigen-specific activation of the T lymphocytes is brought about. However, the NK lymphocytes of Melder have no T-cell receptor. They cannot have the same specificity as the present invention.

Rooney fails to teach autologous lymphocytes. Instead, as discussed in the instant Specification at page 4, line 24 to page 5, line 11, Rooney relates to EBV-specific ***donor-type*** cells. These lymphocytes were obtained from a donor and not from a patient to be treated. See also, Rooney, page 9, Abstract.

Likewise, Wallace also fails to teach autologous lymphocytes. Instead, Wallace teaches EBV-specific cytotoxic T cell lymphocytes obtained from a ***donor***. Wallace also does not teach or suggest utilizing these donor derived cells to treat a virally infected patient.

Although some of the references discuss activating lymphocytes with IL-2, Applicants again direct the Examiner's attention to the teaching away in the Nature Medicine article. As discussed above, the Nature Medicine article discloses that lymphocytes prepared using IL-2 ***were not effective*** in remedying HIV infections, as stated on page 4 of the Specification. Thus, there is clearly no teaching or suggestion in the cited references that activated autologous lymphocytes as recited in the present invention would be effective against viral infections, let alone herpes group viral infections. Consequently, the teachings and suggestions of these references does not create a reasonable expectation of success to one skilled in the art that activated autologous lymphocytes being derived from a culture medium comprising autologous lymphocytes, anti-CD3 antibodies in a solid phase and interleukin-2 would be very effective for herpes group viral infections.

Therefore, the rejection of claims 12-27, 29, and 31-37 under 35 U.S.C. § 103 is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is now in condition for allowance and early notice to that effect is hereby requested.

If it is determined that the application is not in condition for allowance, the Examiner is invited to telephone the undersigned attorney at the number below if he has any suggestions to expedite allowance of the present application.

Respectfully submitted,

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ATTACHMENT TO AMENDMENT AND REPLY:

1. Kawata, Journal of Japan Pediatric Society, Vol. 106, No. 3, pp. 411-412 (2002) with English translation.

2. Ayumi, Journal of Clinical and Experimental Medicine, Vol. 181, No. 6 (May 1997) with English translation.

Journal of Japan Pediatric Society, Vol.106, No.3, pp.411-412 (2002) [Translation]

Application of Activated T-Cell Therapy in Chronic Active EB Virus Infection

Okazaki City Hospital (Pediatrics), Nagoya University (Pediatrics, Medicine Faculty), and Lymphotec Inc.

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[Keywords: "chronic active", "EB virus infection", and "activated T-cell therapy"]

SUMMARY

An activated T-cell therapy was given to a 13-year-old boy with chronic active EB virus infection. On medication, transient increase of EB virus-specific CD8+ cells was observed, resulting in reduction in virus titer and improvement of hepatopathy. The activated T-cell therapy has the potential to effectively treat chronic active EB virus infection.

INTRODUCTION

Chronic active EB virus infection (CAEBV) is a disease developing a chronic or repetitive infectious mononucleosis-like symptom, which lasts through three months or more in a patient having no underlying disease. In this disease, EBV genome having abnormally high anti-EBV antibody value is easily found in the peripheral blood, which is categorized into types of T-cell, B-cell, NK cell. An available therapy for CAEBV has not been established.

The activated T-cell therapy was given to a 13 year-old CAEBV patient, on which immunological and virological analyses were carried out to contemplate availability of the therapy for the disease in question.

CLINICAL CASE

Case Note of Patient: Family history [unspecified]; Previous disease [unspecified]

Disease History: The patient with attach of fever, pharyng-reddening, and erythema multiforme was admitted to a "A" hospital in March 1996 and diagnosed as

infectious monouncleosis. Later, the patient with continuing splenohepatomegaly, pancytopenia and liver dysfunction was exposed to a "B" hospital, and then, introduced to Pediatrics, Medicine Faculty of Nagoya University in March 1998. After careful scrutiny, the patient was diagnosed as CAEBV.

The treatment with vidarabine started in April 1998 effected improvement of hepatopathy and reduction in EBV DNA in the peripheral blood, but was terminated because a side effect of neurologic symptom was produced. Since then, antiviral medications such as acyclovir and ganciclovir were used for treatment, no prominent effect could not be found. Then, chemical therapy using etoposide, predonine and cyclosporine A was performed according to the protocol for treatment of EBV-related hemophagocytic syndrome. Consequently, the benefits of treatment could be passingly recognized, but recurrence was observed two months after. Hence, activated T-cell therapy was started in March 2000 with the approval of the Ethical Committee in Medicine Faculty of Nagoya University.

The results of the medical inspection obtained at starting the therapy revealed that pancytopenia of WBC of 1300/pH, Hb of 11.6 g/dL and Plt $8.5\times104/\mu$L could be recognized. On these occasions, EBV-VCA-IgG (FA) was 1280 times, EBV-EBNA (FA) was 10 times, and EBVDNA was 1402 copies/mL. The peripheral blood mononuclear cells were fractionated by Magnetic Beads to quantitatively determine the amount of EBVDNA, as the result of which infection with NK cells were ascertained.

PROCESS

The activated T-cells were prepared by isolating the mononuclear cells from the peripheral blood of the patient in question, nonspecifically stimulating the isolated cells with solid-phase CD3 antibody, and cultivated the cells in the presence of interleukin-2 (IL-2). The activated T-cells thus obtained were subjected to a bacteriological inspection to ensure the safety thereof, and then, cryopreserved. Just after thawed, activated T-cells were administered to the patient in question in a dose of 10^5/kg. The amount of EB virus-specific CD8+ T-cells thus administered was identified by allowing the peripheral blood of the patient in question to be infected with EBV to prepare lymphoblasoid cell line (LCL) and cultivating LCL and peripheral blood mononuclear cells of the patient in the presence of IL-2 and monensin for 5 hours. Interferon γ yielded as the result of encounter of CD8 T-cells with antigen was measured by use of a flow cytometer. Repetition of the EB virus-specific CD8+ T-cells was represented in the term of proportion of interferon γ positive cells in the CD8 T-cells. The EBV genome was

identified with DNA derived from the peripheral blood mononuclear cells of the patient by using an ABI 7700TaqMAN system.

RESULTS

The disease's and treating courses are shown in FIG. 1. The activated T-cells were administered to the patient 11 times in total in the period from March to August as indicated by the arrows. Viruses have increasingly attritioned out from the beginning of administration and passingly grew in May, but thereafter, were on the wane. Although elevated levels of ATS and ALT were shown about one month after beginning the administration, reaction to the activated T-cells was observed about from the fifth dose to progressively improve ATS and ALT from that time, and conclusively, a stable transition has been kept after July in which the ninth dosage of the activated T-cells was given to the patient, involving in slight regression of splenic tumor and palliation of fever.

The transition of change in EB virus-specific CD8+ T-cells in the peripheral blood is shown in FIG. 2. Where the activated T-cells were given to the patient on 15th day, transitory increase of EB virus-specific CD8+ T-cells could be observed on the following day. It was thought that EB virus-specific CD8+ T-cells were on a decline with relief of symptoms.

CONSIDERATION

The activated T-cell therapy has been administered to a patient of vicious tumor such as liver carcinoma since 1980s and recently tried as treatment for immune deficiency disease and viral infectious disease after a bone marrow transplant. Further, from the standpoint of the fact that some of CAEBV patients respond to the treatment with IL-2 and shows lowering of EBV-specific cytotoxic activity, the activated T-cell therapy is expected as an effective treatment technique. Dr. Morio and others administered the activated T-cell therapy to five CAEBV patients, and produced the desired therapeutic effect in all the cases.

We also administered the activated T-cell therapy to five CAEBV patients and measured with time the amount of EBVDNA and change in EBV-specific CD8 T-cells. Repeated administration of the activated T-cells brought about improvement of clinical symptom and decrease of viruses from 1402 copies/μg DNA to 170 copies/μg DNA. In the meanwhile, it was unidentifiable that the subject therapy produces effects of edging out EBV-infected cells and completely curing CAEBV. The action mechanism of the

activated T-cell therapy has been incompletely understood, i.e. it is uncertain whether the therapeutic effect was brought about due to virus-specific (or tumor antigen-specific) T-cells or activation nonspecific immunity. With administration of the activated T-cells, which was performed this time, temporal increase in EBV-specific CD8 T-cells and decrease in virus amount could been recognized, indicating that the subject activated T-cell therapy has the medical benefit owing to the virus-specific cells.

FIG. 1: Transition of transaminase and BV DNA in activated T-cell therapy

FIG. 2: Change in EBV-specific CD8+ T-cells in activated T-cell therapy

日本小児科学会雑誌　106巻3号　411～412（2002年）

慢性活動性EBウイルス感染症における活性化T細胞療法の応用

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キーワード：慢性活動性EBウイルス感染症，活性化T細胞療法

要　旨

慢性活動性EBウイルス感染症の13歳男児例に対して，活性化T細胞療法を行った．投与後EBウイルス特異的CD8陽性細胞の一過性の増加を認め，ウイルス量の減少や肝障害の改善がみられた．活性化T細胞療法は慢性活動性EBウイルス感染症の有効な治療法となる可能性が示唆された．

はじめに

慢性活動性EBウイルス感染症（以下CAEBV）は，基礎疾患のない患者において3カ月以上持続する慢性，あるいは反復性の伝染性単核症様の症状をきたす疾患である．本疾患ではEBVゲノムが末梢血で容易に検出され，抗EBV抗体価が異常高値を示し，感染細胞の種類からT cell，B cell，NK cell typeに分類される．CAEBVに対しては，いまだ確立された治療法は存在していない．

今回，13歳のCAEBV患者において，活性化T細胞療法を行い，免疫学的，ウイルス学的に解析し，本疾患に対する有用性を検討した．

症　例

症例　家族歴，既往歴に特記事項なし

現病歴　平成8年12月，発熱，咽頭発赤，多形紅斑を認めA病院に入院し，伝染性単核症の診断をうけた．肝脾腫，汎血球減少，肝機能障害が持続したためB病院に紹介されたが，口腔内潰瘍，弛張熱出現し，CAEBVを疑われ，平成10年3月に名古屋大学医学部小児科紹介となり，精査の上CAEBVと診断された．

平成10年4月にビダラビンにて治療を開始し，肝障害の改善，末梢血中のEBV DNAの減少を認めたが，副作用である神経症状が出現したため治療を中止した．その後アシクロビル，ガンシクロビル等の抗ウイルス薬を使用したが，明らかな効果はみられなかった．EBV関連血球貪食症候群治療のプロトコールに準じてエトポシド，プレドニン，サイクロスポリンAによる化学療法を施行し一時的に有効であったが，2カ月後に再燃を認めたため，名古屋大学医学部倫理委員会の承認後，平成12年3月より活性化T細胞療法を開始した．

活性化T細胞療法開始時の検査結果はWBC 1,300/μL，Hb 11.6g/dL，Plt 8.5×104/μLと汎血球減少を認め，AST 659IU/L，ALT 349IU/L，LDH 827IU/Lと肝障害を認めた．また，EBV-VCA-IgG（FA）1,280倍 EBV-EBNA（FA）10倍，EBVDNA量は1,402copies

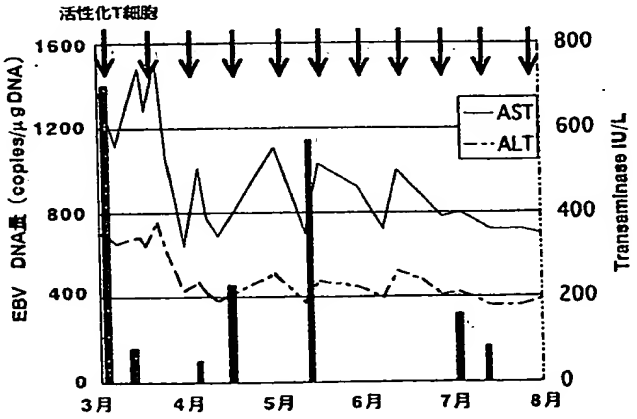

図1　活性化T細胞療法中のEBV DNA量とトランスアミナーゼの推移

（平成13年7月9日受付）（平成13年12月1日受理）
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/μg DNA であった．末梢血単核球を磁気ビーズ法により分画し，EBV DNA 量を定量比較したところ NK 細胞に感染していることが示唆された．

方　法

活性化 T 細胞は患者本人の末梢血から分離した単核球を，固相化 CD3 抗体で非特異的に刺激後，高濃度インターロイキン 2（IL-2）存在下で培養した[1]．得られた活性化 T 細胞を細菌検査などを行い，安全性を確認すると同時に凍結保存し，解凍後速やかに患者に 10^5/kg 静脈内投与した．EBV 特異的 CD8T 細胞の同定は，患者の末梢血に EBV を感染させた lymphoblasoid cell line（LCL）を作成し，次に患者末梢血単核球と LCL を IL-2，モネンシン存在下で 5 時間混合培養した．CD8T 細胞が抗原と出会い産生したインターフェロンγをフローサイトメーターにて測定した．EBV 特異的 T 細胞の頻度は，この INFγ陽性細胞の CD8T 細胞中の割合として表した．EBV ゲノムの定量は患者末梢血単核球より抽出した DNA を ABI 7700TaqMAN system を用いて行った．

結　果

本症例の経過を図 1 に示す．矢印で示したように 3 月から 8 月にかけて活性化 T 細胞を計 11 回投与した．活性化 T 細胞投与開始後，速やかにウイルス量が減少し，5 月に一時増加したが，その後再び減少傾向にある．また，投与開始後約 1 カ月間は AST，ALT は高値を示したが，5 回目頃より活性化 T 細胞に反応し投与ごとに改善が見られるようになり，9 回目の投与を行った 7 月頃より安定して推移している．また，AST，ALT の推移に伴い脾腫の若干の縮小が認められ，発熱回数の減少も観察された．

末梢血中の，EBV 特異的 CD8T 細胞の変化を図 2 に示す．15 日目に活性化 T 細胞を投与したところ，翌日に EBV 特異的 CD8T 細胞の一過性増加が確認された．また，病勢の改善とともに EBV 特異的 CD8T 細胞は減少傾向にあると考えられた．

考　察

活性化 T 細胞療法は，1980 年代から肝癌などの悪性腫瘍の患者に免疫療法として行われてきたが，近年免疫不全症や骨髄移植後のウイルス感染にも試みられ，

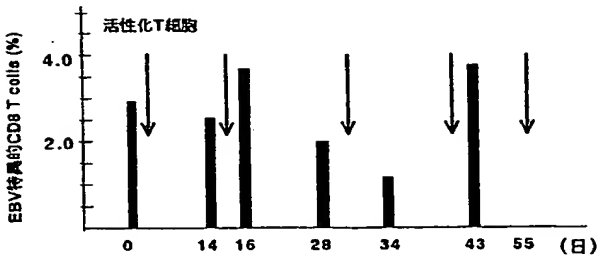

図 2　活性化 T 細胞療法中の EBV 特異的 CD8 陽性 T 細胞の変化

CAEBV 患者の一部が IL-2 による治療に反応を示すことや，EBV 特異的細胞障害活性が低下していることから活性化 T 細胞療法が有効治療として期待されている．森尾らは，5 例の CAEBV 患者に活性化 T 細胞療法を行い，全例で投与効果を認めたとしている[2]．

我々は，CAEBV 患者に活性化 T 細胞療法を投与するとともに，EBVDNA 量および，EBV 特異的 CD8 T 細胞の変化を経時的に測定した．活性化 T 細胞の投与をくり返すことにより，臨床症状が改善し，ウイルス量は 1,402copies/μg DNA から 170copies/μg DNA へと減少した．一方で本治療法により EBV 感染細胞が駆逐される，あるいは CAEBV が完治するまでの効果は得られなかった．活性化 T 細胞療法は，その作用機序については不明な点が多く，治療効果が T 細胞によるウイルス特異的（あるいは腫瘍抗原特異的）な細胞によるものであるのか，非特異的な免疫の附活化によるものなのか明らかではない．今回，活性化 T 細胞の投与に伴い，EBV 特異的 CD8T 細胞の一過性増加，およびウイルス量の減少を認め，活性化 T 細胞療法はウイルス特異的な細胞により効果を発現していることが示唆された．

文　献

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[Translation]

IGAKU NO AYUMI

[Weekly Journal of Clinical and Experimental Medicine]

Vol.181, No. 6, May 10, 1997

MEDICAL TREATMENT OF VIRAL INFECTIOUS DISEASE OF IMMUNOCOMPROMISED PATIENTS BY INFUSION OF EX VIVO ACTIVATED T CELLS

[Keywords: "Wiskott-Aldrich syndrome", "activated lymphocyte infusion", "solid-phase CD3 antibody" and "IL-2"]

The Wiskott-Aldrich syndrome (WAS) is an immune deficiency disease involving a sex-linked recessive heredity pattern, characterized by immunity deficiency showing hemorrhagic tendency due to thrombocytopenia, refractory eruption, and cell mediated immunity. The immunity deficiency is a bad prognosis, which becomes severe with age, to often cause malignant tumor serious enough to result in death for a patient before he or she is 10 years old. The normal treatment for this disease is a bone marrow transplant as a current first-line treatment, but it is difficult to find a related consanguineous donor. Thus, treatment with an unrelated-donor bone marrow transplant has commonly been administered, but has fundamental difficulties in assuring a bone marrow donor and problems of DVHD and various chronic infections. Therefore, the conventional treatment has not always been satisfactory.

There have been reported that the WAS immune abnormality is malfunction of cellular immunity possibly resulting in a decline in absolute number of T cells, lowering of lymphocyte blastogenesis to mitogen, disturbance of T-cell activation, and disappearance of microvilli on lymphocyte surface, and such symptoms develop with age. Lately, WAS protein has been identified to proceed observational study of its function.

■ Treatment with lymphocytes activated by solid-phase CD3 antibody and IL-2

Dr. Sekine and others developed a method for increasing lymphocytes by 1000 times or more in two weeks by activating peripheral blood lymphocytes with solid-phase CD3 antibody and IL-2 and cultivating the activated lymphocytes by using a gas-permeable bag. This method makes it possible to easily prepare 10^{10} level of activated lymphocytes from 20 ml of blood collected. The midterm report of a clinical comparative investigation on hepatocellular carcinoma using the aforenoted method revealed that recurrence of hepatocellular carcinoma in patients administered with the

activated lymphocytes could be significantly curtailed without causing a serious side effect such as vascular leak syndrome as found in a LAK therapy except for mild fever, cephalalgia, and sicchasia. The authors applied to control viruses by activating ex vivo the lymphocytes from the patients in WAS cases with disorder of activation of T cells, and proliferating the activated lymphocytes.

■ Activated lymphocyte infusion treatment to WAS patients

An activated T-cell infusion treatment was given to a 10 year old boy, who had herpes simplex and EB viral persistent infection and became harder to control the condition of the disease with antiviral drugs. The activated lymphocytes of ① 2.8×10^9, ② 2.7×10^9, ③$3.2\times10^9$, and ④ 8.8×10^9 were infused four times into the patient every three to four days. CRP was transferred to negative one week after infection, and herpes eruption disappeared three weeks after infection, as shown in FIG. 1. Even more amazingly, refractory eruption was in remission, and no side effect with infection was produced.

■ Change in immunity owing to administration of activated lymphocytes

Analyses of lymphocyte blastogenesis to PHA and ConA, IL-2 competence, and secular change in lymphocyte subsets were conducted to evaluate the indicator of immunity of the patient. EB virus transformed B-cell line obtained from the peripheral blood of the patient was measured as a target cell with time to observe cytotoxic T lymphocytes (CTL) to EB virus-infected cells. Simultaneously, semi-quantitative analysis for determining the number of EB virus-infected cells in the peripheral blood of the patient was carried out by a PCR hybridization method. The lymphocyte blastogenesis to PHA and ConA of the patient before administration of the activated lymphocytes was remarkably lower in level than that of a normal healthy person, but after administration, the lymphocyte blastogenesis rose to the level of the normal healthy person. Also, the IL-2 competence was increased as well after administration of the activated lymphocytes to the patient. Increases of the absolute number of T-cells and the ratio among CD8, CD3/HLA and DR positive cells were measured after administration the activated lymphocytes to the patient. As shown in FIG. 3, CTL activation to EB virus was induced in vivo after administration the activated lymphocytes, thus decreasing EB virus-infected cells in the peripheral blood of the patient.

In addition to our own clinical experiments demonstrated hereinabove with respect to the medical treatment of WAS by using the activated lymphocytes, studies on dose and dosing interval of the activated lymphocytes are now being carried on. The studies

have revealed that improvements of symptom and immune function occurred in two clinical cases by repeatedly administrating the activated lymphocytes at intervals of two to four weeks. Besides, it was found that the required amount of activated lymphocytes can be obtained from only about 20 ml of blood collected from the patient, and further, administration of the activated lymphocytes produces little side effects, as the result of which the medical treatment using the activated lymphocytes can be effectively applied to young child patients. The proposed treatment capable of inducing virus-specific cytotoxic T lymphocyte (CTL) is found to be useful as a symptomatic therapy for remedying infection anathema of the WAS patient during the waiting period until bone marrow transplant. The treatment in question has potential for treating primary immunity deficiency and acquired immune deficiency syndromes. The authors are now preparing for putting the treatment to clinical applications. The authors would express special appreciation to Dr. Noboru Okuno, of Pediatrics of Asahikawa Medical University, for the assistance and support given to us.

FIG. 1: Activated lymphocyte infusion treatment to WAS patients
Prominent herpes eruption and refractory eruption before infection (left photo) disappeared three weeks after infection (right photo).

FIG. 2 Change in lymphocyte blastogenesis with time
On analyses of lymphocyte blastogenesis to PHA and ConA, lymphocyte blastogenesis before administration of the activated lymphocytes was remarkably lower in level than that of a normal healthy person (n=200), but after administration, the lymphocyte blastogenesis rose continuously for a certain period.

FIG. 3 CTL activity and NK activity to EBV transformed cell
As the result of 4-h ^{51}Cr release test conducted using peripheral blood of the patient as a target cell of EB virus transformed B-cell line, CTL to EB virus-infected cells was induced in vivo after administration of the activated lymphocytes, but no change in NK activity was found, allowing supposition that restrictive HLA-specific immune reaction was carried out.

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●あゆみ

アンジオテンシンII受容体とその拮抗薬

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アンジオテンシンII受容体拮抗薬の薬理学的性質
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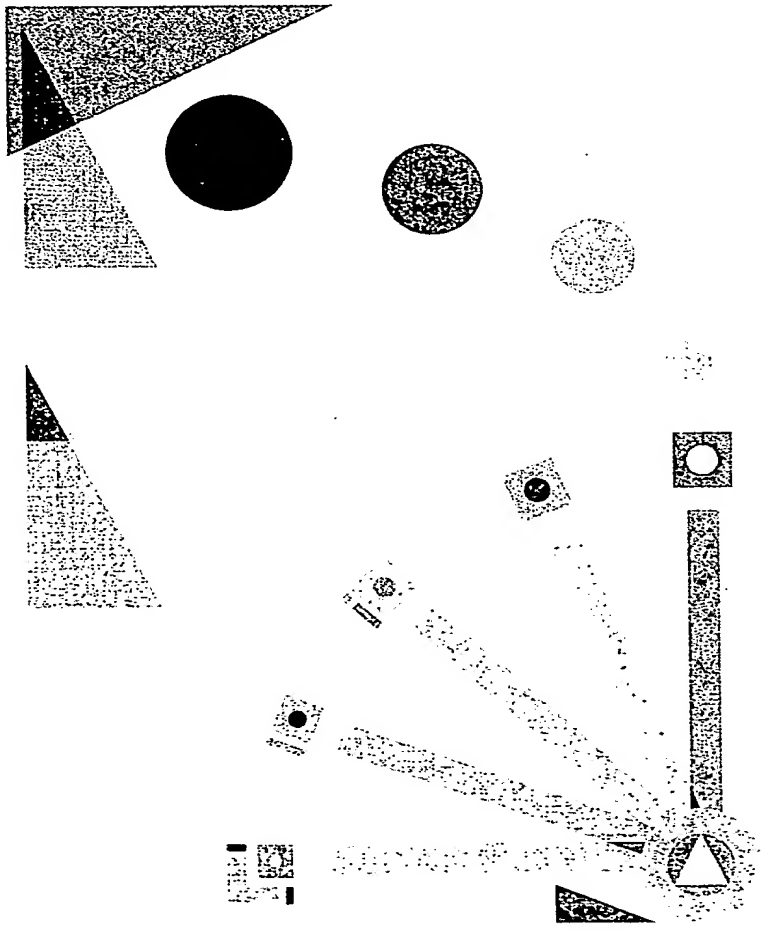

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話題

ex vivo 活性化T細胞の輸注による免疫不全患者に対するウイルス感染症の治療

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キーワード：Wiskott-Aldrich 症候群，活性化リンパ球輸注剤，固相化 CD 3 抗体，IL-2

Wiskott-Aldrich 症候群（WAS）は，血小板減少による出血傾向，難治性湿疹，細胞性免疫を中心とした免疫不全を主徴とする疾患で，伴性劣性遺伝形式をとる疾患である[1]．免疫不全は年齢とともに重症化し，多くは10歳までに悪性腫瘍を発症し死亡する予後不良な疾患である．治療は現在のところ骨髄移植が第一選択とされるが，遺伝性疾患のため血縁者のドナーが得られにくく，非血縁者間での移植が中心となるが，ドナーの確保やGVHD，また多くは慢性の感染症を有しているため移植のタイミングなど問題点も多く，かならずしも満足のいく成績は得られていないのが現状である．

WASの免疫異常は細胞性免疫の機能異常としてT細胞の絶対数の低下，マイトゲンに対するリンパ球幼若化反応の低下，T細胞の活性化障害，リンパ球表面のmicrovilliの消失などが報告されており，加齢によりその程度は進行する．最近，WAS蛋白が同定されその機能の解析が進められている．

■固相化 CD 3 抗体と IL-2 による活性化リンパ球療法

関根らは，固相化CD 3抗体とIL-2を用いて末梢血リンパ球を活性化させ，さらにガス透過性バッグを用いた培養法により2週間で1,000倍以上に増幅する方法を開発した[2]．この方法を用いれば約20 m*l*の採血量で，10^{10}レベルの活性化リンパ球を容易に得られるのが特徴である．この方法を用いた肝細胞癌における臨床比較試験の中間報告では，活性化リンパ球投与群で有意に再発が抑えられた．しかも副作用は，軽度の発熱，頭痛，嘔気がみられたほかは，LAK療法で認められるvascular leak syndromeのような重篤なものはなかった[3,4]．著者らは，T細胞の活性化障害のみられるWAS症例で患者リンパ球を*ex vivo*で活性化，増幅し，ウイルスのコントロールに応用した．

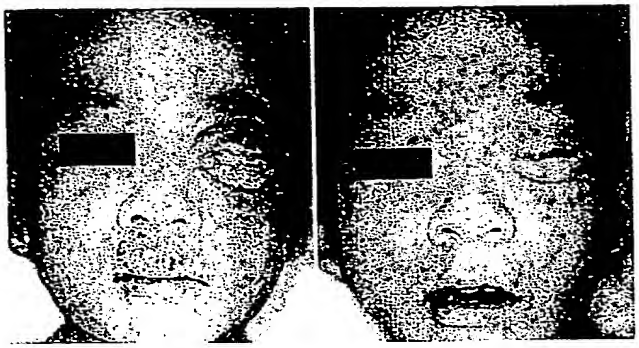

図1　WAS 患者への活性化リンパ球輸注療法
輸注前（左）に著明だったヘルペス疹と難治性湿疹は，輸注後3週間目（右）に消失した．

■WAS 患者への活性化リンパ球輸注療法

患者は11歳の男児で単純ヘルペスおよびEBウイルスの持続感染をきたし，抗ウイルス剤でのコントロールは困難となってきたため活性化T-cell輸注療法を開始した．輸注プロトコールは，①$2.8\times10^9$個，②$2.7\times10^9$個，③$3.2\times10^9$個，④$8.8\times10^9$個の活性化リンパ球を3〜4日ごとに4回輸注した．輸注後1週間でCRPは陰転化し，3週間で図1に示すようにヘルペス疹は消失した．また，驚くことに難治性湿疹も軽快した．輸注に伴う副作用は認められなかった．

■活性化リンパ球投与による免疫能の変化

患者の免疫能の指標としてPHA, ConAに対するリンパ球幼若化反応，IL-2反応能，リンパ球サブセットの経時変化を解析した．また，患者末梢血より樹立したEB virus transformed B-cell lineを標的細胞として，EBウイルス感染細胞に

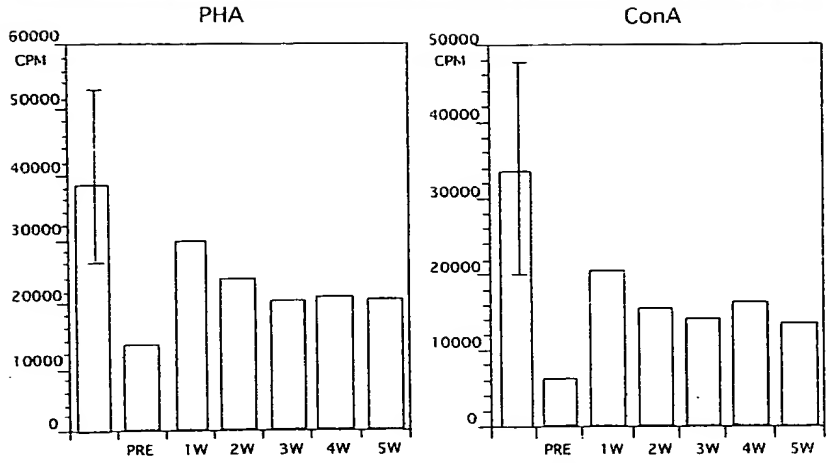

図2　リンパ球幼若化反応の経時変化

PHA および ConA に対するリンパ球幼若化反応を検討した．輸注前はグラフ左端の健常成人（$n=200$）に比べて低値であったが，活性化リンパ球投与後はリンパ球幼若化反応は上昇し，かつ一定期間持続した．

対する細胞障害活性（CTL）を経時的に測定した．同時に患者末梢血中の EB ウイルス感染細胞数の半定量を PCR hybridization 法により解析した．輸注前は，図2に示すように PHA，ConA に対するリンパ球幼若化反応は健常人に比べ，著しい低値を示したが，輸注後健常人のレベルまで上昇した．同様に IL-2 反応能も活性化リンパ球輸注後は上昇した．リンパ球サブセットは，輸注後 T 細胞の絶対数の増加と CD 8，CD 3/HLA DR 陽性細胞の比率が増加した．また，図3で示すように活性化リンパ球輸注後 *in vivo* で EB ウイルスに対する CTL 活性が誘導され，患者末梢血中の EB ウイルス感染細胞は減少した．

以上，WAS に対する活性化リンパ球療法の自験例を紹介した．現在リンパ球の投与量と投与間隔の検討も行っているが，同患者で明らかに免疫機能と臨床症状の改善がみられる期間は 2〜4 週で繰返しの投与が必要であった．しかし，約 20 m*l* の採血で投与に必要な活性化リンパ球が得られ，しかも副作用がほとんどないことから，小児患者でも安全に施行できること，ウイルスに対する CTL が誘導できる点で WAS 患者の骨髄移植までの待機期間あるいは感染増悪に対する対症療法として期待できる治療と思われる．また，原発性

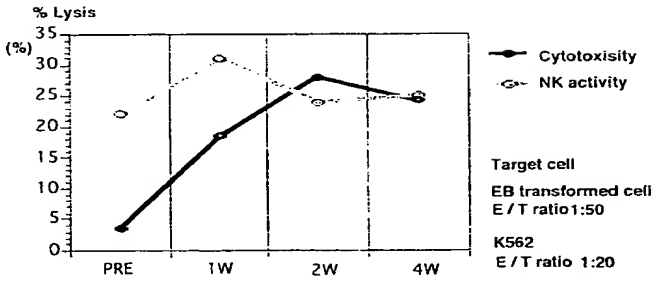

図3　EBV transformed cell に対する CTL 活性と NK 活性

target cell として患者末梢血より樹立した EBV transformed B cell，effector cell として患者末梢血リンパ球を用い，^{51}Cr 放出試験（4時間法）を行った．活性化リンパ球輸注後，*in vivo* で EBV に対する CTL が誘導された．一方 NK 活性は変化なく，HLA 拘束特異免疫反応が誘導されたことが推測される．

免疫不全のほか後天性免疫不全症候群においても効果が期待できると考えられ，現在臨床応用の準備を進めている．

本稿を書くにあたって御指導いただいた旭川医科大学小児科教授奥野晃生先生に感謝いたします．

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